identical. Thus, there is a less than 2% difference in doubling time between all 96 strains. The primers used for amplification of the tags were ATCCTACAACCTCTCTAG and TACCCATTCTAACCTCTA.
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17. To regenerate the mutant, we digested the plasmids with Eco R1 and used them to transform strain BG14. The genomic DNA flanking YipLac211 directed homologous recombination back to the original locus, thus regenerating the original insertion mutant.
18. The *EPA1* sequence has been deposited in GenBank (accession number AF149048)
19. Hep2 monolayers were fixed with 2% paraformaldehyde. Surface carbohydrates were disrupted by incubation in the dark at room temperature with 10 mM sodium metaperiodate in 50 mM sodium acetate (pH 4.5) for 1 hour. Monolayers were washed twice in PBS and reduced with 50 mM sodium borohydride (50 mM in PBS) for 30 min at room temperature. Monolayers were rinsed with PBS and used in adherence assays. Control monolayers were treated identically with the exception of the sodium metaperiodate. Yeast cells treated with periodate were not fixed, and washes were carried out in microcentrifuge tubes with centrifugation at 500*g* (*32*).
20. CHO cells and their derivatives were cultured in α-MEM with 10% FBS. The adherence assays were carried out identically to those with HEp2 cells.
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23. The vaginal candidiasis model we used is a modification of one described for *C. glabrata* in the literature (*22*). In our modification, we administered two doses of estrogen, one 5 days and one 3 days before infection. These doses increase the overall CFUs recoverable to 10^5 to 10^6 per animal at day 6 and decreases mouse-to-mouse variability in colonization. Subsequent to these initial doses of estrogen, no more is administered. The GI infection model is a colonization model in which 6- to 7-week-old DBA2 mice are given 10^8 organisms by oral inoculation. By day 3, the most substantial site of colonization in the GI tract was the stomach, consistent with what is seen in *C. albicans* infections. About 1 to 5×10^4 CFUs persisted at this site for 9 days. By day 15 after infection, the recoverable CFUs had dropped to about 10^3 per animal.
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36. pEPA1 was made by cloning the Hind III—Eco RI fragment of *EPA1* [which includes the coding region and 3′ untranslated region as well as 54 nucleotides upstream of the ATG] into the *S. cerevisiae* expression vector p416TEF (*33*). Adherence of two independent transformants was tested in triplicate.
37. We thank P. Fidel and J. Sobel for the gift of the *C. glabrata* clinical isolate; K. Clemons and D. Stevens for the clinical *S. cerevisiae* isolates; E. Ortega-Barria for many helpful discussions and advice; and J. Mecsas, C. Greider, and S. Fisher for a critical reading of the manuscript. This work was funded by a grant to S.F. from SmithKline Beecham. B.P.C. was supported by a postdoctoral fellowship from the Helen Hay Whitney Foundation and a career award from the Burroughs Wellcome Fund.

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Transcriptional Activation of APETALA1 by LEAFY

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Plants produce new appendages reiteratively from groups of stem cells called shoot apical meristems. LEAFY (LFY) and APETALA1 (AP1) are pivotal for the switch to the reproductive phase, where instead of leaves the shoot apical meristem produces flowers. Use of steroid-inducible activation of LFY demonstrated that early expression of *AP1* is a result of transcriptional induction by LFY. This *AP1* induction is independent of protein synthesis and occurs specifically in the tissues and at the developmental stage in which floral fate is assumed. Later expression of *AP1* appears to be only indirectly affected by LFY.

The above ground body plan in higher plants is generated postembryonically by a group of undifferentiated stem cells, the shoot apical meristem (SAM). Initially, the *Arabidopsis thaliana* SAM produces leaves with axillary, second-order shoot meristems. At the transition to the reproductive phase, the primary shoot switches to the production of flowers. Two meristem identity factors, LEAFY (LFY) and APETALA1 (AP1), are necessary and sufficient for this transition (*1*–*4*). Severe disruption of the onset of reproduction is observed in the loss-of-function *lfy-6* mutant; most flowers are replaced by leaves and second-order shoots (*3*). In the strong *ap1-1* mutant, flowers have partial shoot character (*1*). The gain-of-function phenotype produced by constitutive expression of either LFY or AP1 results in formation of flowers or leaves and flowers in positions normally occupied by leaves and second-order meristems (*2*, *4*). The LFY protein localizes to the nucleus, and LFY binds to a putative *AP1* promoter element in vitro (*5*). AP1 is a potential transcriptional target of LFY because it acts, in part, downstream of LFY (*1*–*7*). Moreover, *AP1* expression is delayed and reduced in *lfy* mutants [our data and (*2*, *8*–*10*)], whereas constitutive ectopic expression of LFY results in precocious *AP1* expression (*5*). However, these data do not allow separation of direct transcriptional activation by LFY from downstream effects that influence gene expression. To test whether LFY acts as a transcriptional activator in vivo, we constructed a steroid hormone–inducible, posttranslational LFY switch (*11*). This switch allows us to monitor the immediate effect of LFY activation on transcription, in the presence of translational inhibitors.

We transformed plants segregating for the sterile *lfy-6*–null mutation with a constitutively expressed translational fusion of LFY to the rat glucocorticoid receptor hormone binding domain [35S::LFY-GR (*12*)]. Using antiserum to LFY (*13*), we identified several lines expressing full-length fusion protein (Fig. 1A). The amount of LFY-GR protein detected in the nuclei increased after dexamethasone hormone treatment (*14*) (compare Fig. 1C with Fig. 1B). Thus, activation of the fusion protein results in proper subcellular localization of LFY-GR (Fig. 1D) (*5*, *15*).

In *lfy-6*–null mutants, floral organs that require the expression of the class B homeotic genes (petals and stamens) are absent (*3*, *16*). To test whether the fusion protein is biologically active, we followed the development of *lfy-6* 35S::LFY-GR flowers after dexamethasone treatment. As expected, petals and stamens were also absent in untreated *lfy-6* 35S::LFY-GR flowers (Fig. 1E). This defect was partially or fully rescued after hormone treatment (Fig. 1, F and G, respectively). In addition, hormone treatment of seedlings resulting from an outcross of *lfy-6* 35S::LFY-GR to the *Ler* wild type reproduced characteristic LFY gain-of-function phenotypes (*4*) in that second-order shoots were converted to flowers (Fig. 1, H and I). Similarly, treatment of *lfy-6* 35S::LFY-GR seedlings caused conversion of secondary shoots to flowers (*17*). These data demonstrate that the LFY switch we constructed is functional.

To test whether LFY acts as a transcriptional activator in vivo, we monitored *AP1* expression in *lfy-6* inflorescences after LFY-GR activation using in situ hybridization (*14*). Early *AP1* expression in the wild type is first observed in young stage 1 (*18*) flowers immediately after the transition to flowering (Fig. 2H) (*19*). By contrast, *AP1* is absent from stage 1 flowers in *lfy-6* inflorescences immediately after the transition to flowering (compare Fig. 2,

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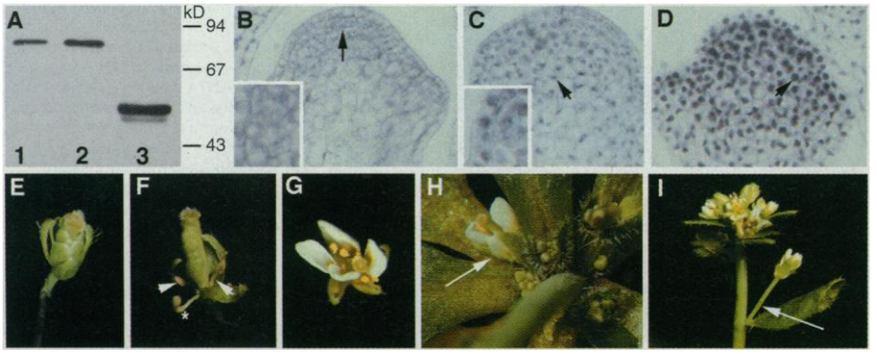

Fig. 1. A biologically active LFY switch. Immunochemical detection of the LFY-GR protein on immunoblot (**A**) and in fixed tissue (**B** to **D**) with polyclonal antibody to LFY (*13*). (A) The full-length fusion protein is overexpressed in inflorescence extracts. The genotypes tested were *lfy-6* 35S::LFY-GR (lanes 1 and 2) and 35S::LFY (lane 3). Equal volumes of crude extract were loaded. Plants were untreated. For immunolocalization, 17-day-old *lfy-6* 35S::LFY-GR seedlings were mock-treated (0.1% ethanol) (B) or treated with 5 μM dexamethasone (Sigma) in 0.1% ethanol (*14*) (C). Insets show cells of (B) and (C) at higher magnification (1400-fold). (D) Immunolocalization of untreated 17-day-old 35S::LFY seedlings. Arrowheads point to cells showing background (B) or increased levels (C and D) of staining for the LFY protein in nuclei. Original magnification, ×320 (B) and ×480 (C and D). (**E**) A mature flower in untreated *lfy-6* 35S::LFY-GR plants consists of sepals and carpels, lacks petals and stamens, and is infertile. (**F** and **G**) *lfy-6* 35S::LFY-GR inflorescences were treated with 5 μM dexamethasone after bolting as described (*20*), and flowers were observed 7 days after treatment. (F) Partially rescued *lfy-6* 35S::LFY-GR flowers have sepal-stamen mosaic organs (asterisk) as well as some stamenoid organs (arrowheads). (G) Fully rescued *lfy-6* 35S::LFY-GR flowers have a normal complement of all floral organ types: four sepals, four petals, six stamens, and two fused fertile carpels. (**H** and **I**) Seedlings of the genotype *lfy-6*/+ 35S::LFY-GR or +/+ 35S::LFY-GR were treated at day 7 (H) and day 10 (I) by a single, 2-hour submersion in 5 μM dexamethasone, and the phenotypes were observed after bolting. (H) An axillary inflorescence meristem acquired floral fate and generated a single flower (arrow). (I) A secondary inflorescence meristem adopted floral fate and generated a single flower, which is subtended by a specialized leaf (bract). Mock-treated inflorescences and seedlings, as well as dexamethasone-treated plants not expressing the LFY-GR fusion protein, were indistinguishable from untreated siblings.

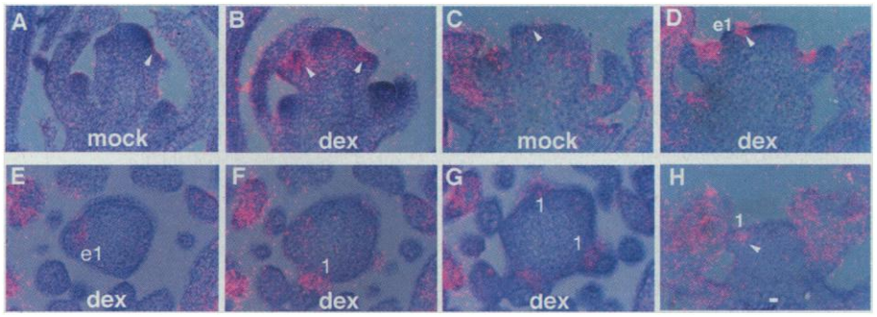

Fig. 2. Early *AP1* expression in response to LFY activation in *lfy-6* 35S::LFY-GR. Presented are representative results of eight independent induction experiments analyzed by four separate in situ hybridization experiments. Tissues were fixed, sectioned, and hybridized as described (*29*). Developmental stages of plant and specifics of the hormone treatment are described (*14*). (**A** and **B**) Longitudinal sections of *lfy-6* 35S::LFY-GR seedlings. (**C** and **D**) Longitudinal sections of *lfy-6* 35S::LFY-GR inflorescences. (**E** to **G**) Serial transverse sections of *lfy-6* 35S::LFY-GR inflorescences. Young axillary inflorescences and seedlings were treated with solution without hormone (mock) (A and C) and 5 μM dexamethasone (dex) (B, D, and E to G). Plants were treated twice, at time 0 and again after 4 hours. They were harvested 6 or 8 hours after the first treatment and processed immediately. Sections in (A) and (B) as well as those in (C) and (D) and those in (E) to (G) are from the same in situ hybridization slides and are therefore fixed, probed, exposed, and developed identically. (**H**) Wild type was not treated (−). Early stage 1 (e1) and stage 1 (1) flowers are indicated throughout. Arrowheads point to sites of early *AP1* expression. The inflorescence architecture is different in young *lfy-6* meristems compared with wild-type meristems as all floral primordia in the *lfy-6* mutants arise in the axils of bracts. Dexamethasone treatments of seedlings without the LFY-GR fusion protein showed AP1 expression indistinguishable from mock-treated seedlings. Original magnification, ×160.

C and H) (*2*, *9*, *10*). After hormone induction in *lfy-6* 35S::LFY-GR plants at the floral transition (*14*), we detected *AP1* RNA in early stage 1 primordia (Fig. 2D) as well as in stage 1 primordia that are formed in the axils of bracts (Fig. 2, B and E to G). This expression was not observed in mock-treated siblings (compare Figs. 2, A and B, and 2, C and D). Thus,

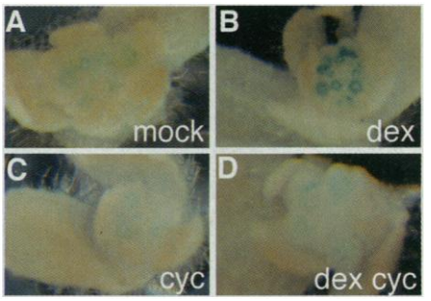

Fig. 3. Increased GUS activation in AP3::GUS plants in response to dexamethasone only in the absence of protein synthesis inhibitors. Young axillary inflorescences of *lfy-6* 35S::LFY-GR AP3::GUS plants (*14*) were mock-treated (**A**) or treated with 5 μM dexamethasone (dex) (**B**), 10 μM cycloheximide (cyc) (**C**), or 5 μM dexamethasone plus 10 μM cycloheximide (dex cyc) (**D**). GUS assays were performed as described (*30*). Original magnification, ×20.

activation of LFY-GR in *lfy-6* mutant plants causes rapid changes in *AP1* mRNA toward the expected wild-type expression pattern. These changes occur in the tissues and at the developmental stage when floral fate is first assumed, suggesting that LFY might act as a transcriptional activator of *AP1*.

To be able to determine whether AP1 induction occurs independently of protein synthesis, we first retested our procedure for cycloheximide inhibition of protein synthesis (*20*) in the 35S::LFY-GR genotype. Protein synthesis–dependent GUS staining was analyzed in an AP3::GUS transgenic line, where the LFY-regulated AP3 promoter (*21*) controls the production of the RNA for a bacterial beta-glucuronidase (GUS). GUS staining increased when *lfy-6* 35S::LFY-GR inflorescences (*14*) were treated with dexamethasone alone but not when cycloheximide was applied together with dexamethasone (compare Fig. 3B with Fig. 3, A, C, and D), indicating that protein synthesis is abolished efficiently. This finding is further supported by experiments with a heat shock–inducible promoter fused to the GUS reporter in seedlings and inflorescences (*20*) (see supplementary figure, available at www.sciencemag.org/feature/data/1040108.shl).

We next tested whether *AP1* is an immediate target of LFY in vivo by analysis of *AP1* expression after LFY activation in the absence of protein synthesis. We observed *AP1* induction in young *lfy-6* 35S::LFY-GR flower primordia (Fig. 4, C and D) in the presence of hormone and cycloheximide. This induction was not observed when only cycloheximide was used (compare Figs. 4, A and B, with Fig. 4, C and D). Thus, LFY activation in *lfy-6* mutants results in early expression of *AP1* independent of new protein synthesis, indicating that AP1 is a direct transcriptional target of LFY.

After the transition to flowering, *AP1* was

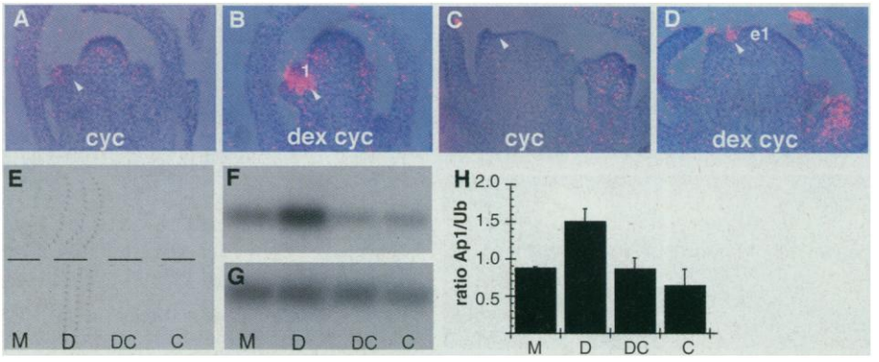

Fig. 4. AP1 is an immediate target of LFY. Experimental setup is as described in Fig. 2. Longitudinal sections of seedlings that were treated with (**A** and **C**) 10 μM cycloheximide (cyc) or (**B** and **D**) 5 μM dexamethasone plus 10 μM cycloheximide (dex cyc). Sections in (A) and (B) as well as those in (C) and (D) are from the same in situ hybridization slides. Original magnification (A to D), ×160. (**E**) Autoradiograph of two representative in situ slides after overnight exposure. (**F** to **H**) RT-PCR analysis of five axillary inflorescences treated with solution without hormone (M), 5 μM dexamethasone (D), 5 μM dexamethasone plus 10 μM cycloheximide (DC), and 10 μM cycloheximide (C). (F) RT-PCR with AP1 primers. (G) RT-PCR with ubiquitin primers (*14*). PCR products were subjected to electrophoresis on agarose gels and after transfer to nylon membranes were probed with the labeled PCR product. Triplicate experiments were quantitated with a phosphorimager (H). The mean of the AP1 values corrected by normalization with the ubiquitin (Ub) lanes and their standard error are shown.

expressed in *lfy*-null mutants in more mature stage 3 flowers (Figs. 2C and 4C), although the expression level was reduced compared with the wild type (*2*, *9*, *10*). This later *AP1* expression is thus quantitatively dependent on LFY but does not require LFY activity. We measured *AP1* expression in entire inflorescences, which monitor primarily the more abundant later *AP1* expression, using both semiquantitative in situ hybridization and reverse transcription followed by polymerase chain reaction (RT-PCR) (Fig. 4, E to H). We detected a general increase of *AP1* mRNA levels after LFY activation in hormone-treated compared with mock-treated *lfy-6* 35S::LFY-GR plants. Unlike the *AP1* induction in early flower primordia, this general increase was only observed when no protein synthesis inhibitor was present (Fig. 4, E to H), suggesting that this response is controlled indirectly by LFY (*22*).

Using inducible LFY activity, we were thus able to show that AP1 is an immediate target of transcriptional activation by LFY. This finding is consistent with the observed temporal and spatial expression pattern of both genes. *AP1* is expressed later and within the *LFY* expression domain until stage 3 of flower development (*3*, *5*, *19*, *23*–*25*). However, after photoinduction and activation of upstream regulators, lag times of up to 56 hours have been observed between the onset of *LFY* and *AP1* expression (*23*, *24*). These lag times could be explained by postulating that a critical threshold level of LFY is required for *AP1* induction. Consistent with this notion, *LFY* expression increases during vegetative development (*26*). A second possibility, not mutually exclusive with the first, is that a function independent of LFY marks time or developmental stage, ensuring that *AP1* induction by LFY occurs at the right time and place in development. A similar model appears to be true for targets of the floral homeotic gene *APETALA3* (*20*). In support of this second hypothesis, we did not detect *AP1* induction using in situ hybridization when we activated LFY in *lfy-6* 35S::LFY-GR plants before the floral transition (*14*, *17*).

We conclude that LFY acts as a transcriptional activator and that it exerts its meristem identity activity in part by direct transcriptional activation of AP1.

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11. This system allows us to posttranslationally activate the fusion protein without nonspecific effects on the plant because the fusion protein is held in the cytoplasm until hormone treatment and because the treatment itself has no apparent effects on *Arabidopsis* [our data and (*20*, *24*, *27*)].
12. 35S::LFY-GR consists of the LFY open reading frame [amino acids 1 to 424 (*3*)] fused to the rat glucocorticoid receptor (amino acids 508 to 795) with overlapping PCR primers and subcloned into pCGN18 as described in (*20*). The LFY coding region was introduced into pCGN18 to create 35S::LFY. Both proteins carry a 13–amino acid NH_2-terminal extension consisting of a FLAG epitope and a HMK site. Both constructs were introduced into the *Ler* ecotype of *Arabidopsis*. 35S::LFY exhibited the same gain-of-function phenotypes as previously described (*3*). AP3::GUS was created by transformation of *Ler* with the plasmid described in (*28*) (J. L. Riechmann and E. M. Meyerowitz, unpublished data).
13. Polyclonal antibodies against LFY were produced in rabbits with recombinant hexa-histidine–tagged LFY protein after Nickel-NTA column (Qiagen, Santa Clarita, CA) purification and size separation on denaturing gels. Crude serum was affinity purified against recombinant glutathione S-transferase–tagged LFY protein. Later bleeds of the crude serum were indistinguishable from the affinity-purified antibody and were used at 1:2000 dilution on immunoblots and at 1:6000 dilution in immunolocalization experiments. Immunolocalization was as in (*15*).
14. Plants were grown at 18°C in continuous light. For immunolocalization and GUS analyses, plants were treated as described for the in situ analyses. In situ protocols were previously described (*29*). The probes used were as in (*19*) (*AP1*) and (*3*) (*LFY*). To monitor *AP1* induction at the transition to flowering, we removed the central shoot and treated young axillary inflorescences before visible bolting. In addition, 15- to 17-day-old seedlings were treated, and after sectioning, primary shoots that had just started to initiate flowers were mounted for in situ hybridization. For LFY-GR activation before the floral transition, seedlings were 8 or 11 days old. Inflorescences and seedlings were treated twice, at time 0 and again after 4 hours. They were harvested 6 or 8 hours after the first treatment and processed immediately. The hormone or protein synthesis inhibitor, or both, was applied to inflorescences in ethanol and 0.015% Silwet L-77 (OSI Specialties, Danbury, CT) for increased penetration, and seedlings were submerged in hormone or protein synthesis inhibitor, or both, in 0.1% ethanol. The primers used for RT-PCR were as follows: AP1 5′, GCAATGAGCCCTAAAGAGCTTCAG; AP1 3′, CATGTAAGGATGCTGGATTTGGTGC; UB 5′, GGTGCTAAGAAGAGGAAGAAT; and UB 3′, CTCCTTCTTTCTG-GTAAACGT. Products were in the linear range of the response as determined by phosphorimager quantitation (with 22 cycles for AP1 and 20 cycles for ubiquitin amplification).
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22. These findings suggest that after the initial induction of *AP1* by LFY, full levels of *AP1* expression require either the presence of a preexisting, unstable protein or de novo protein synthesis. Alternatively, prolonged inhibition of protein synthesis (*14*) may cause depletion of general transcription machinery proteins and explain the failure to see high levels of *AP1* expression in the presence of cycloheximide.
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